FIG.1

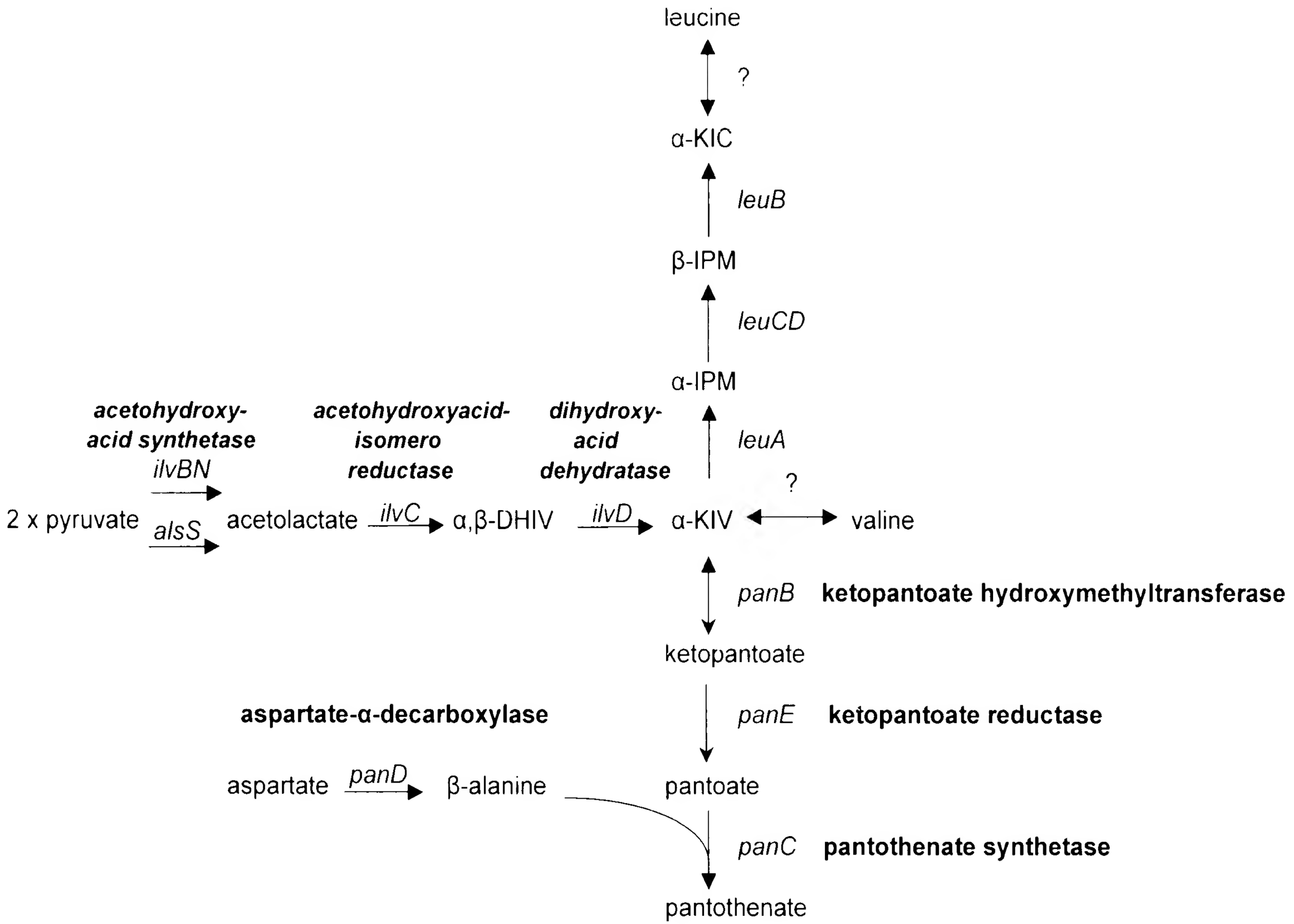

leucine
?
α-KIC
leuB
β-IPM
leuCD
α-IPM
acetohydroxy-acid synthetase
ilvBN
acetohydroxyacid-isomero reductase
dihydroxy-acid dehydratase
leuA
?
2 x pyruvate
alsS
acetolactate
ilvC
α,β-DHIV
ilvD
α-KIV
valine
panB
ketopantoate hydroxymethyltransferase
ketopantoate
aspartate-α-decarboxylase
panE
ketopantoate reductase
aspartate
panD
β-alanine
pantoate
panC
pantothenate synthetase
pantothenate

Figure 2. Plasmid pAN240, containing sequences ligated upstream of the P_{26}**panBCD** *cassette, equivalent to the integrated version in strain PA221.*

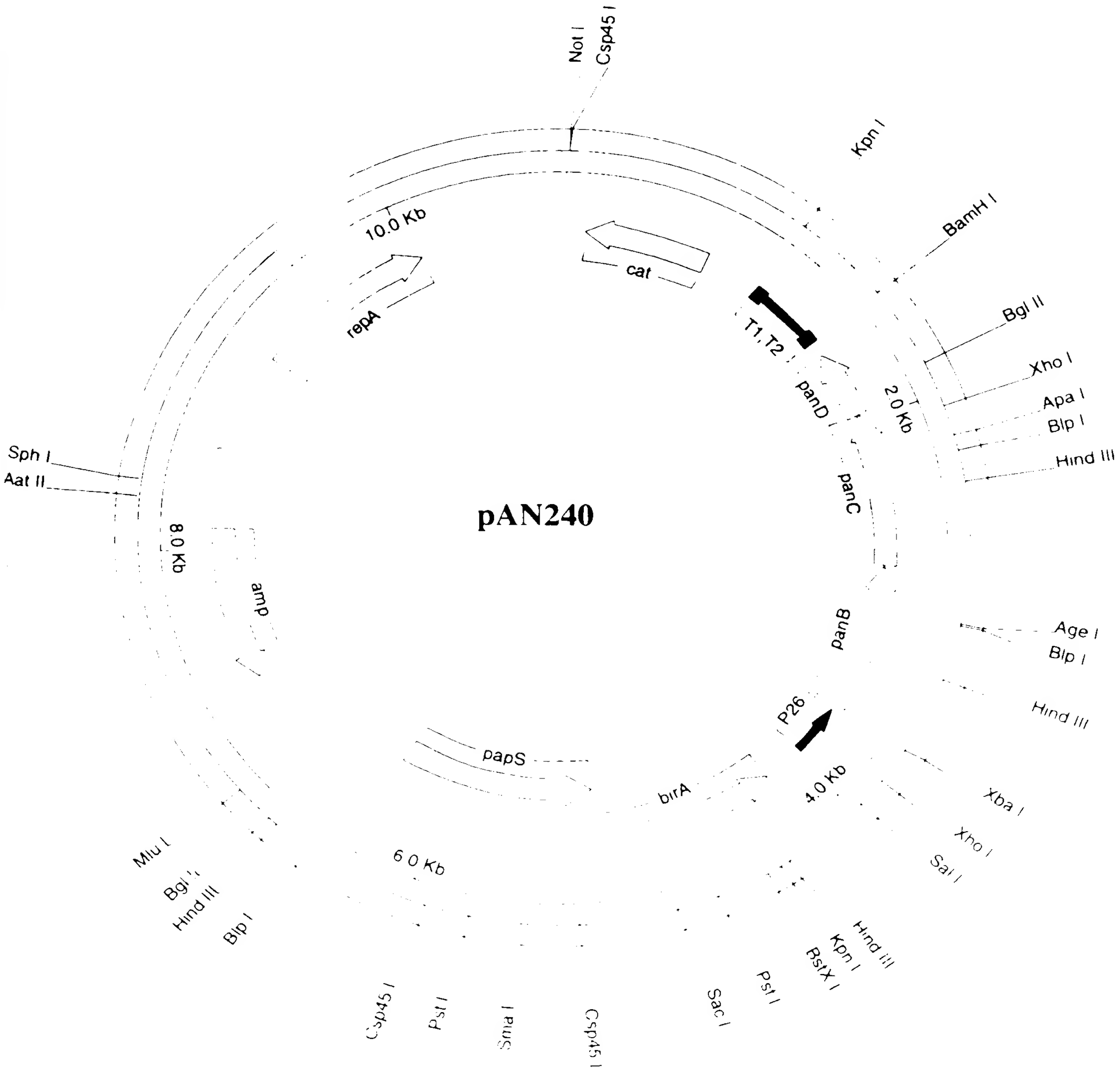


Figure 3A Plasmid pAN004, containing the panBCD *operon expressed from P26 and RBS1.*

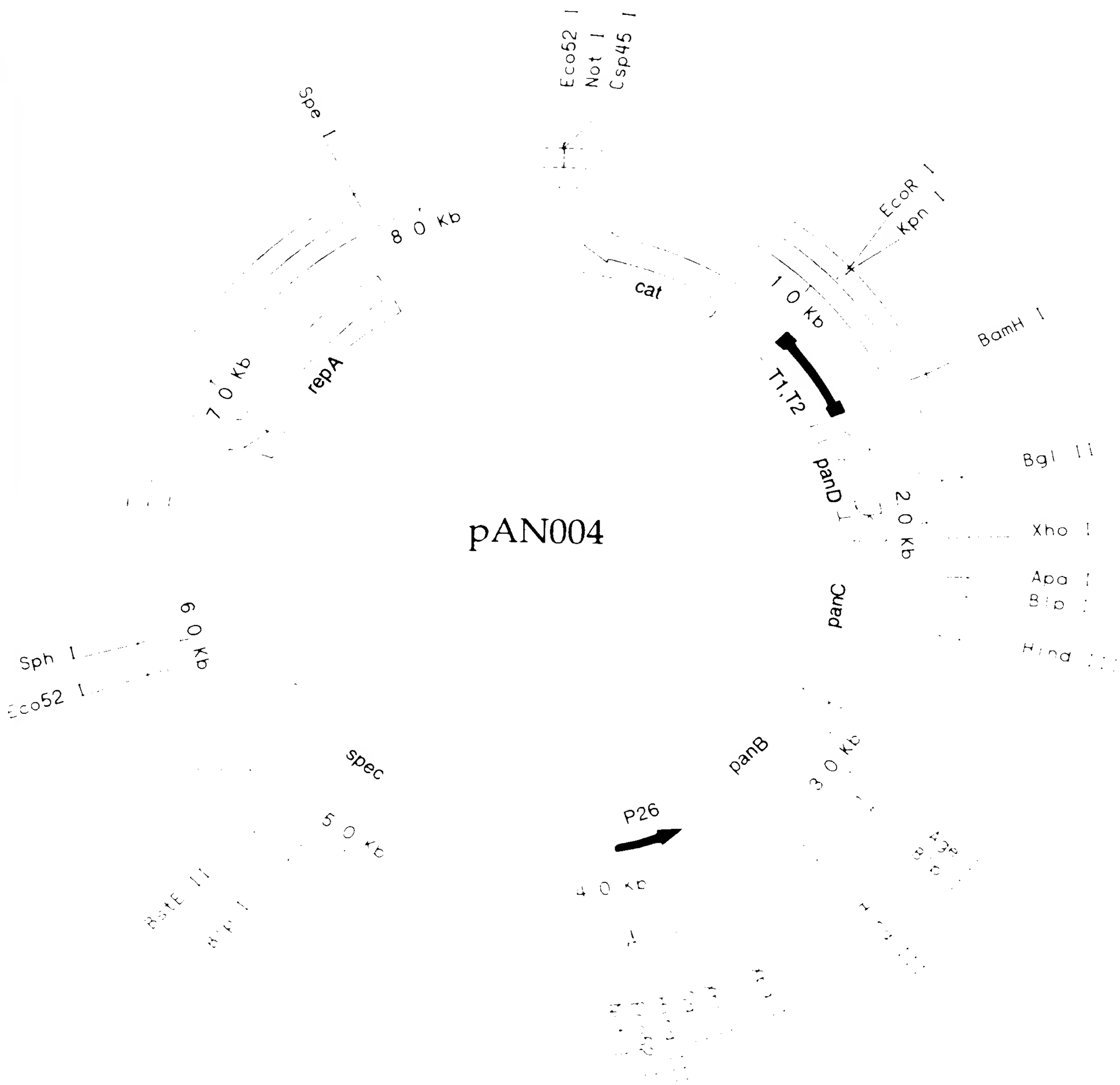


Figure 35 Plasmid pAN006, containing the panBCD *operon expressed from P26 and RBS2.*

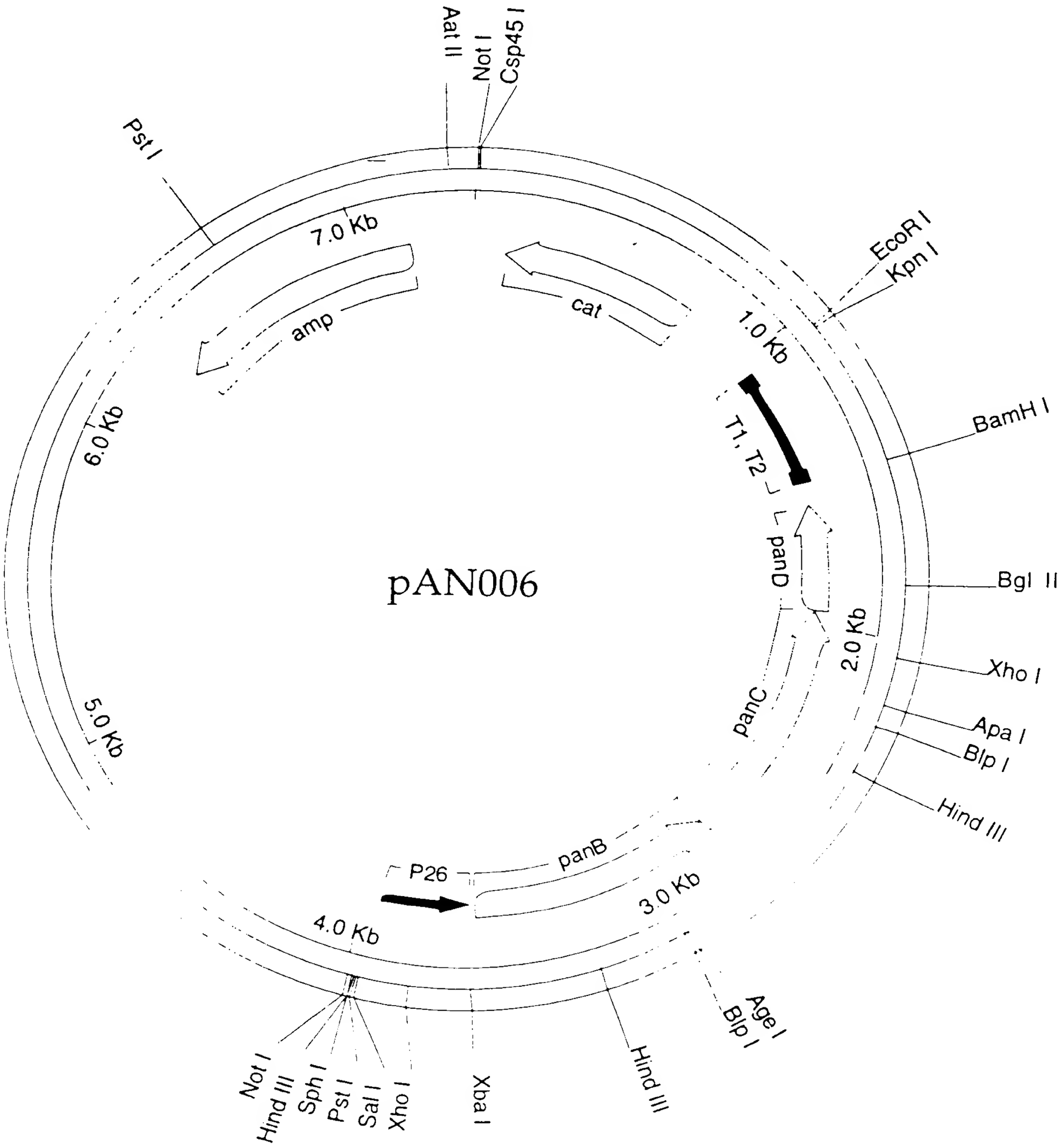


Figure 4 Plasmid pAN236, containing an integratable and amplifiable P26-RBS2-panE1 expression cassette.

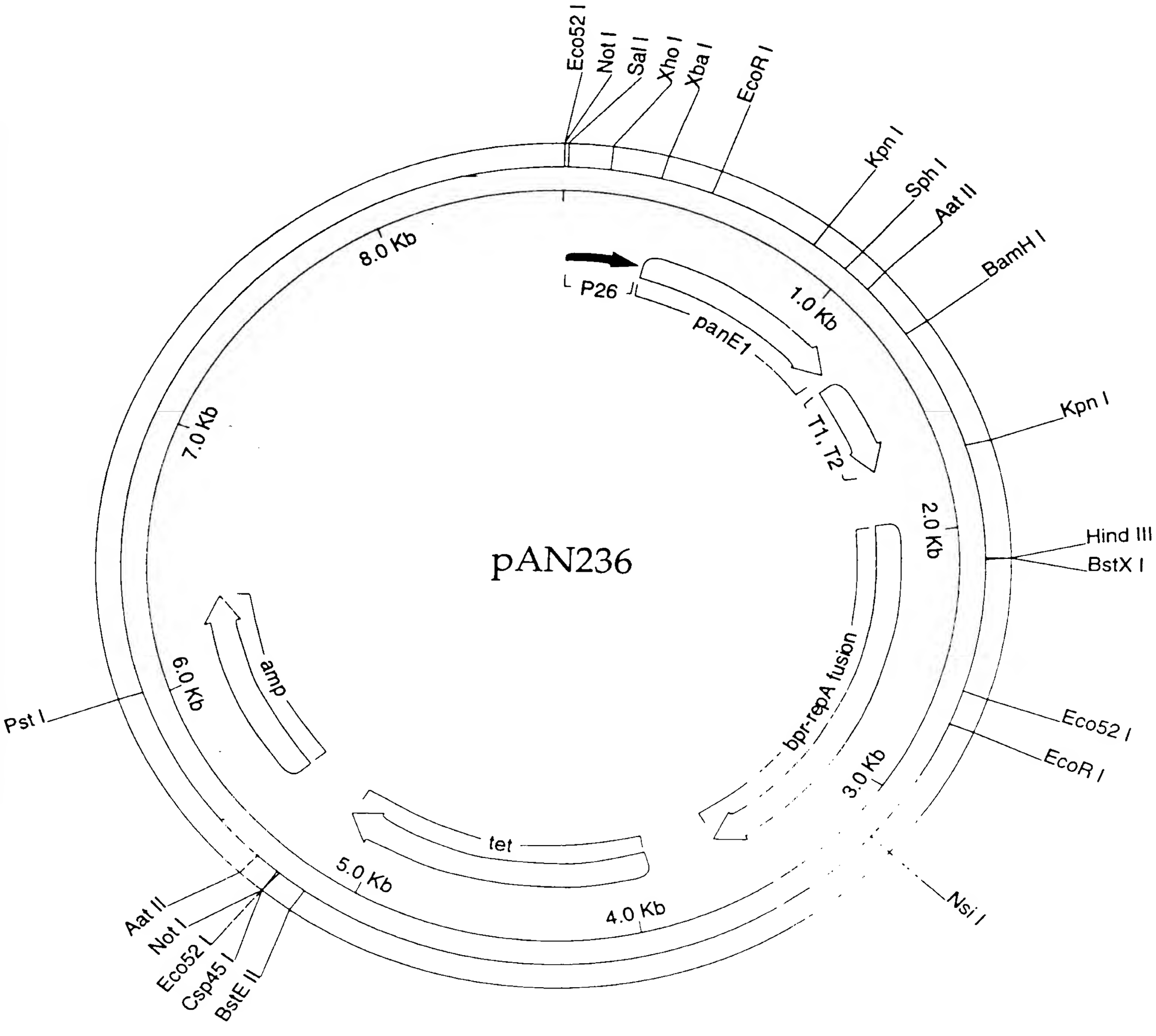


Figure 5 Construction of pAN423

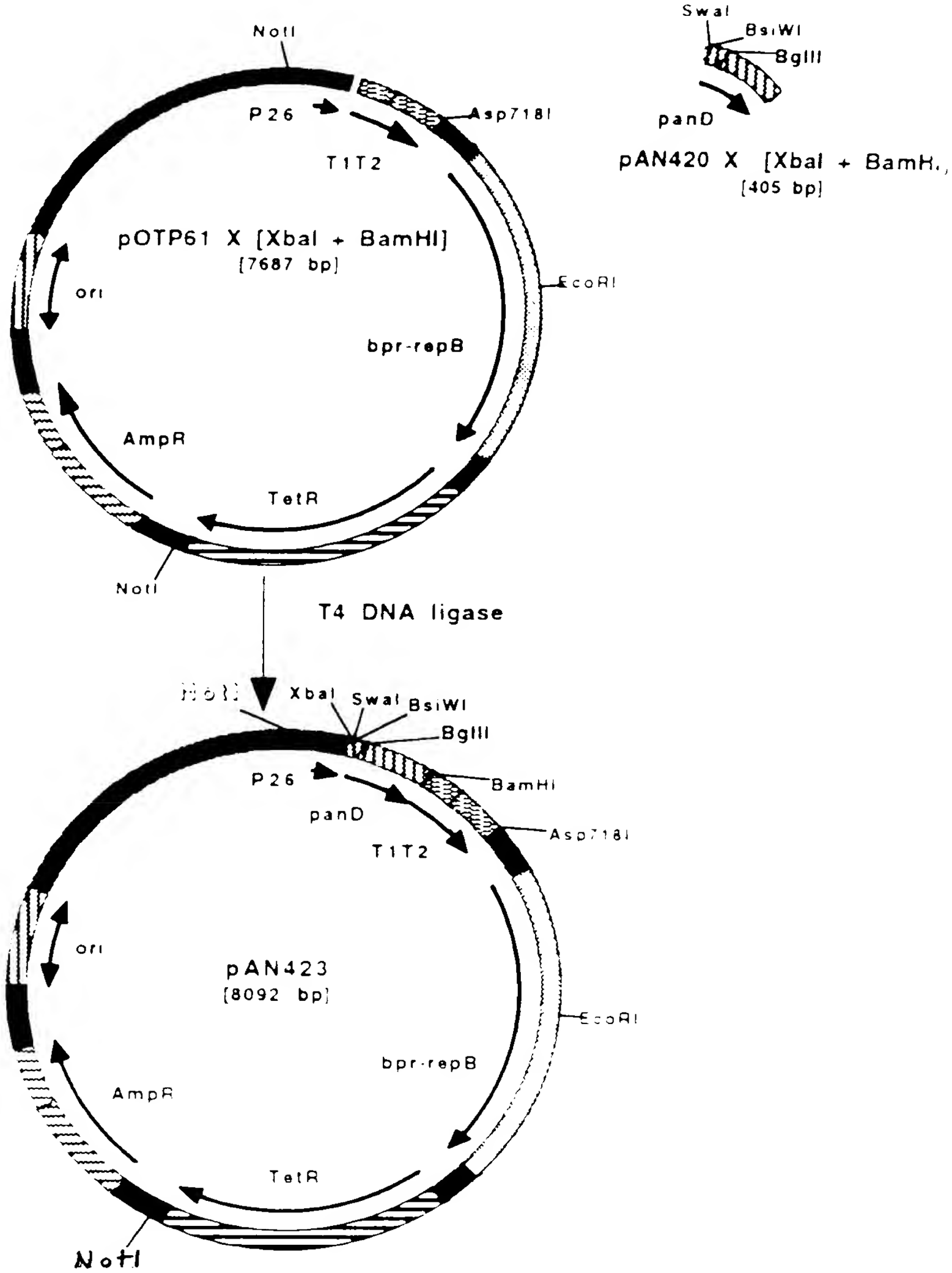


Figure 6 Construction of pAN426 and pAN427.

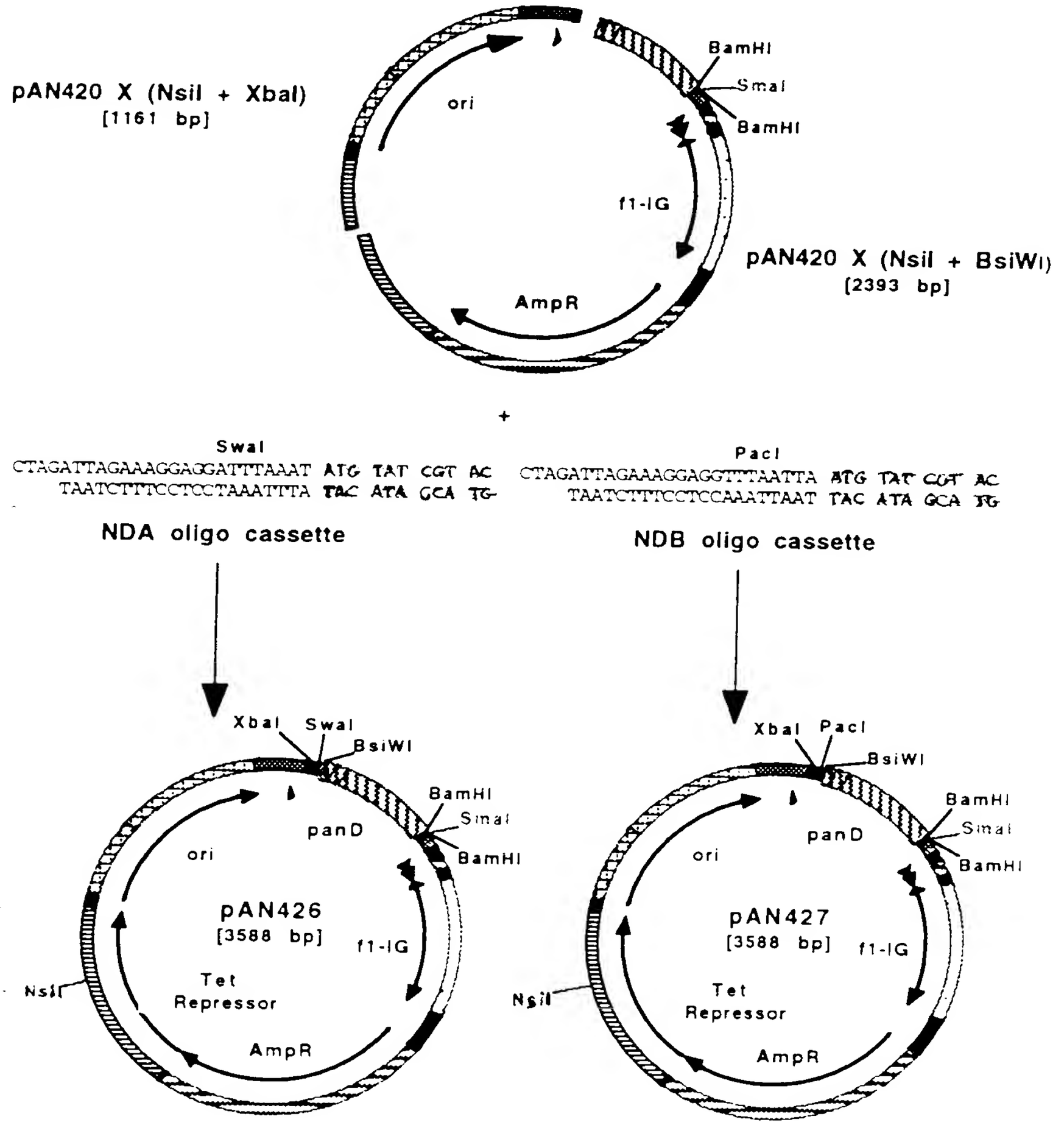


Figure 7 Construction of pAN428 and pAN429.

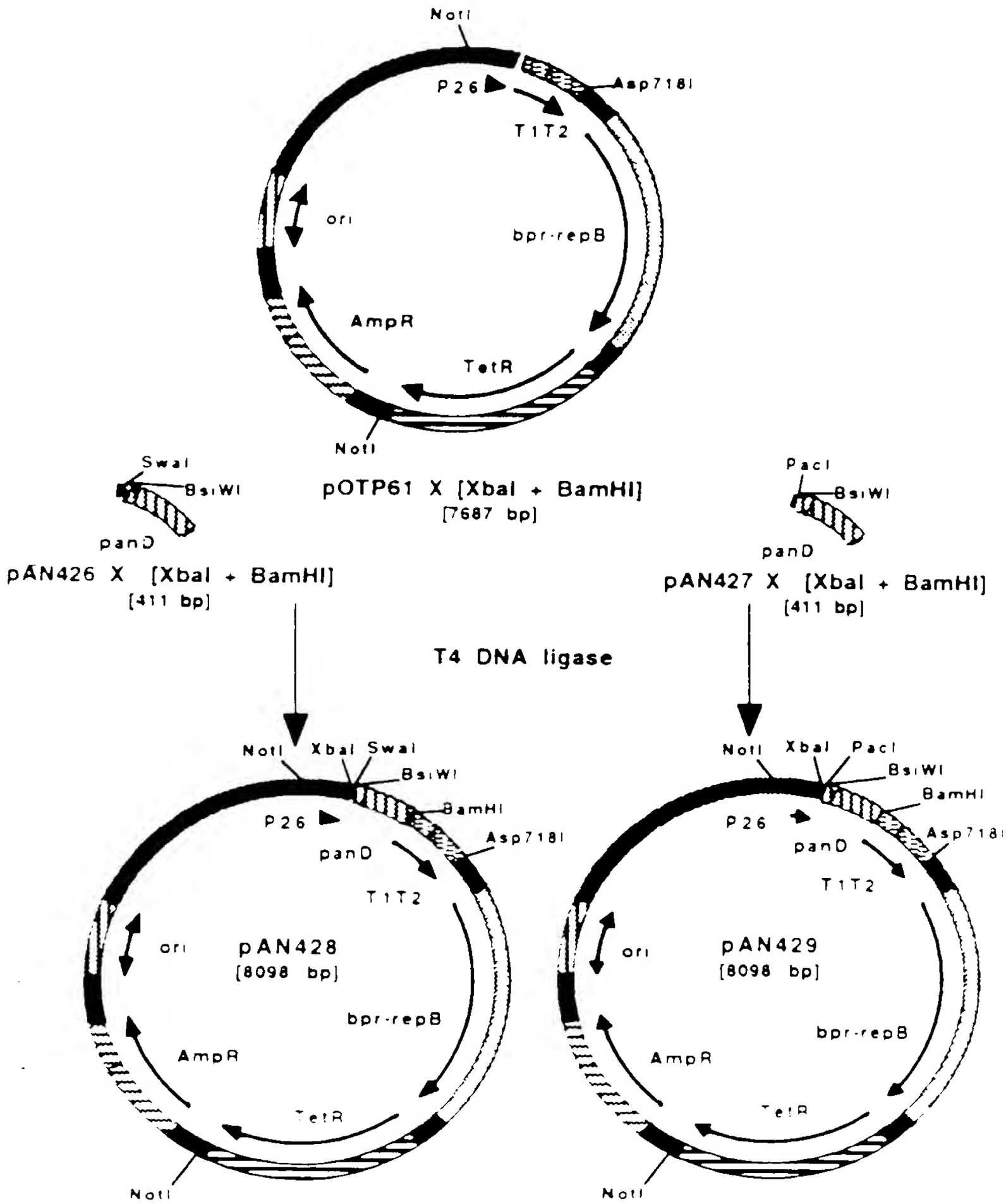
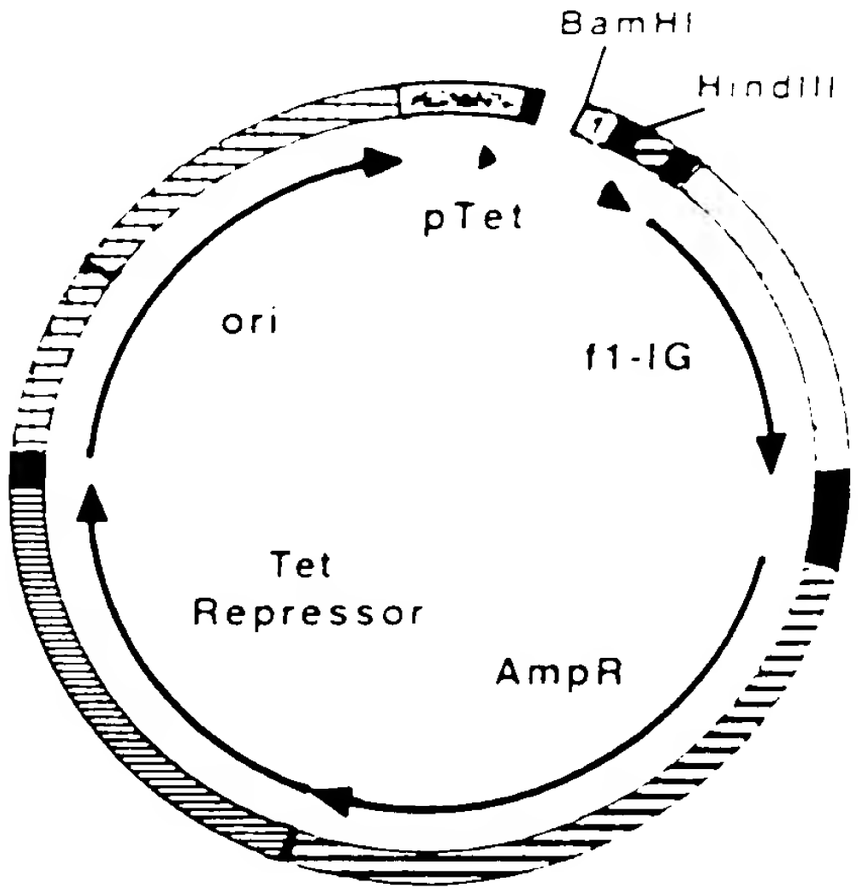
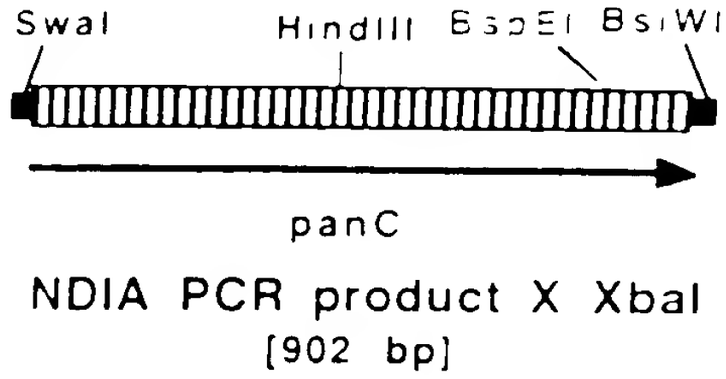


Figure 8. Construction of pAN431.



pASK-1BA3 X [XbaI + SmaI]
[3169 bp]



NDIA PCR product X XbaI
[902 bp]

T4 DNA ligase

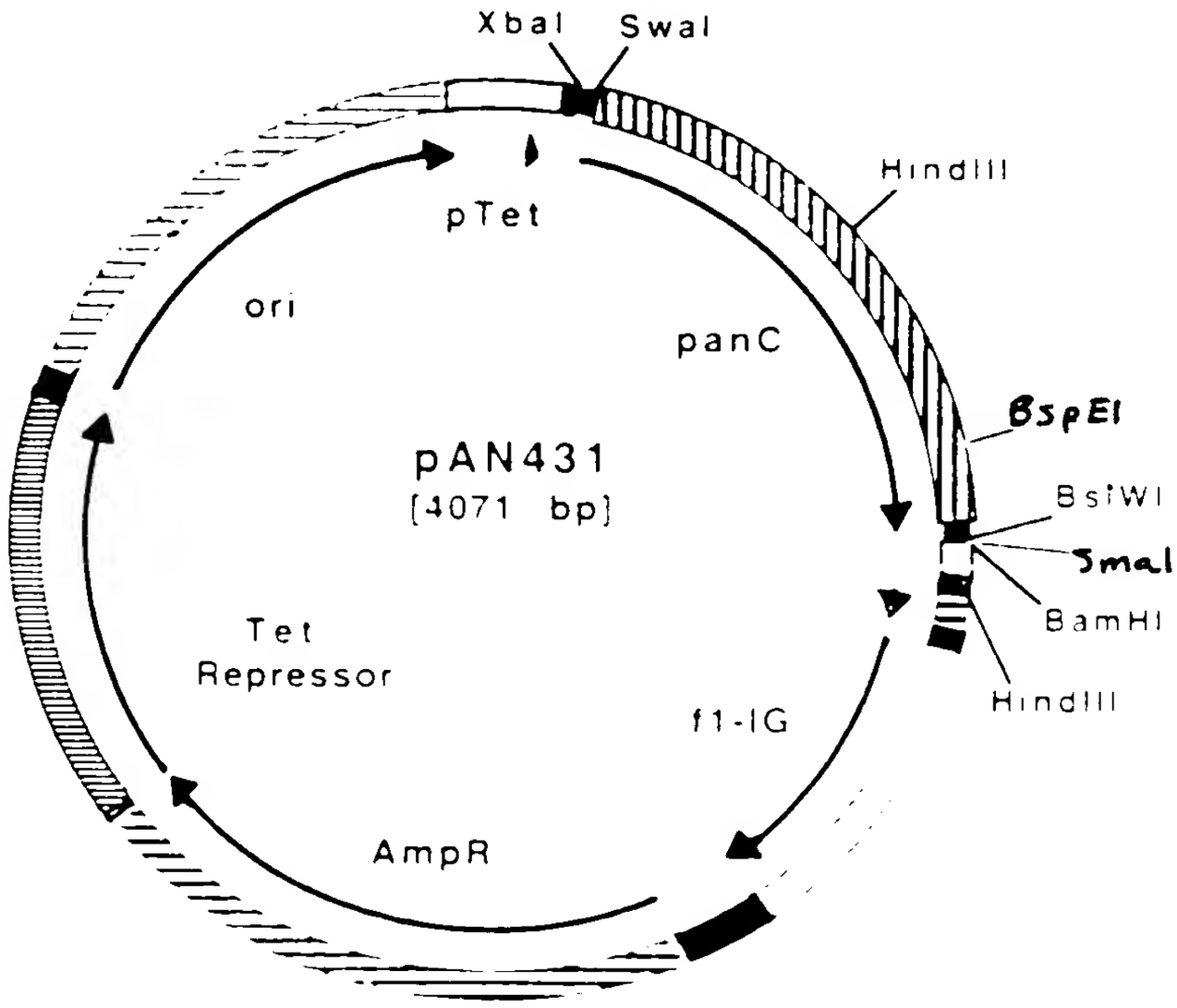


Figure 9. Construction of pAN441.

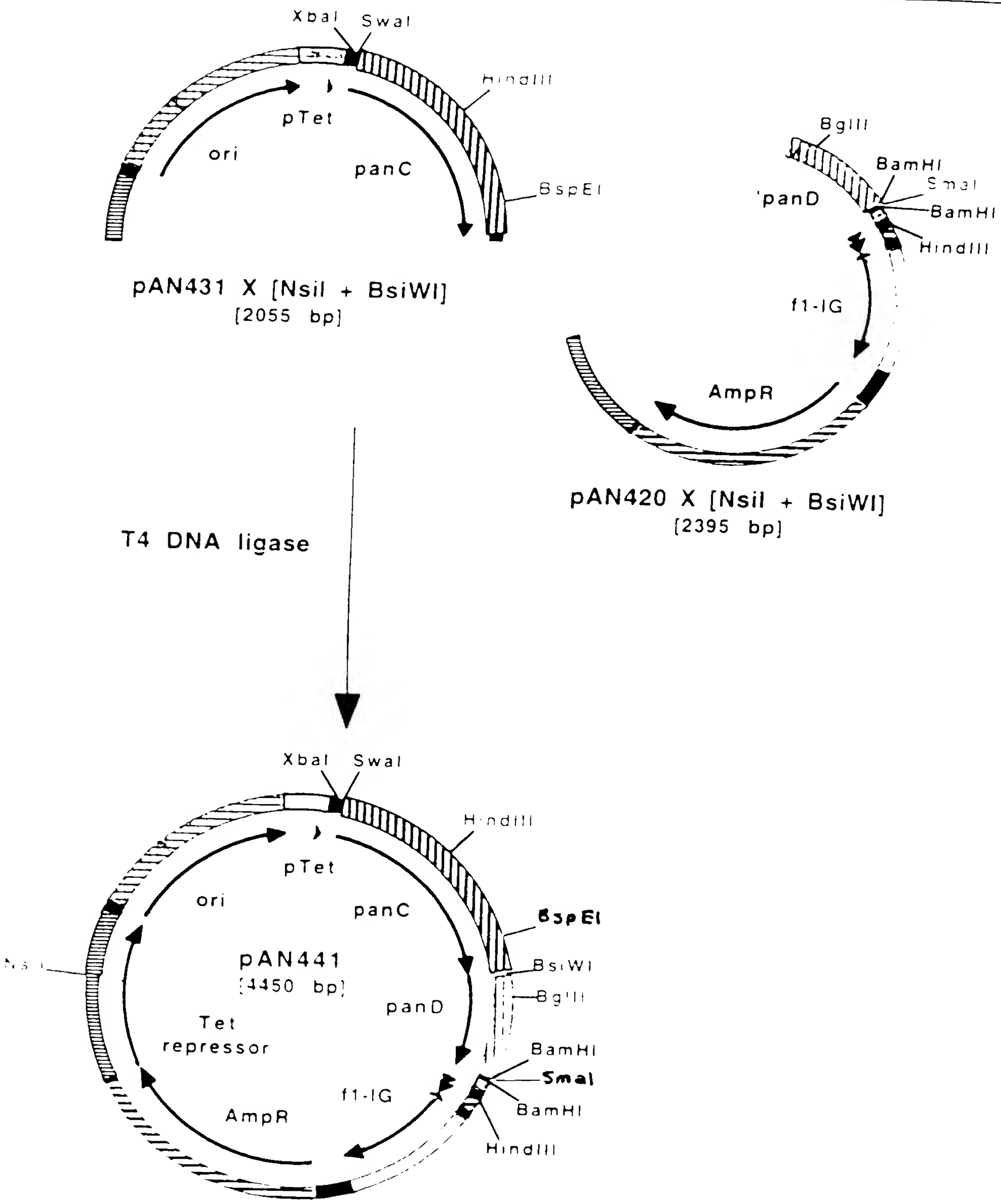


Figure 10. Construction of pAN440.

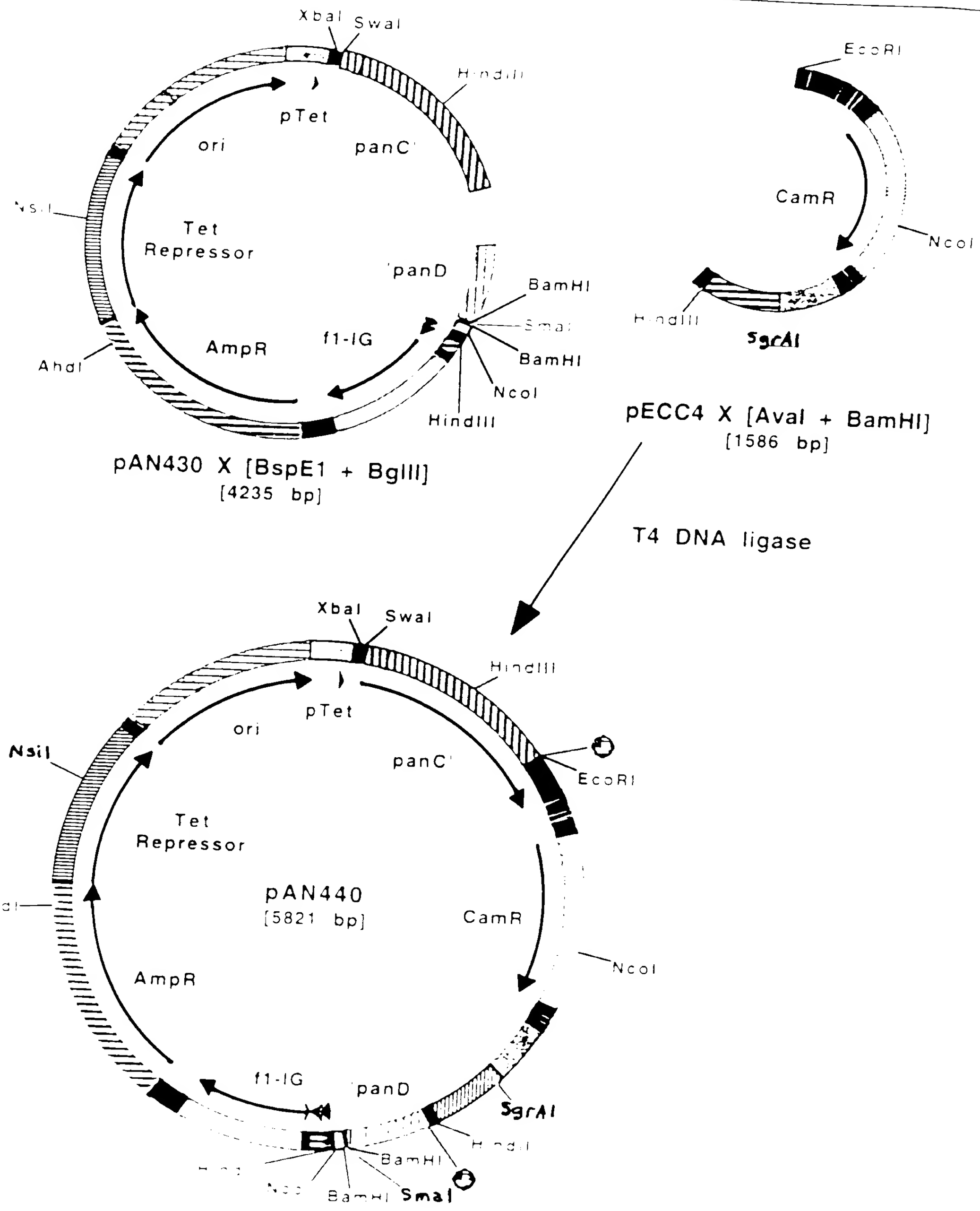


Figure 11 Structure of pAN251, a plasmid designed to integrate a single copy of P_{26} panE1 *at the* panE1 *locus by double crossover.*

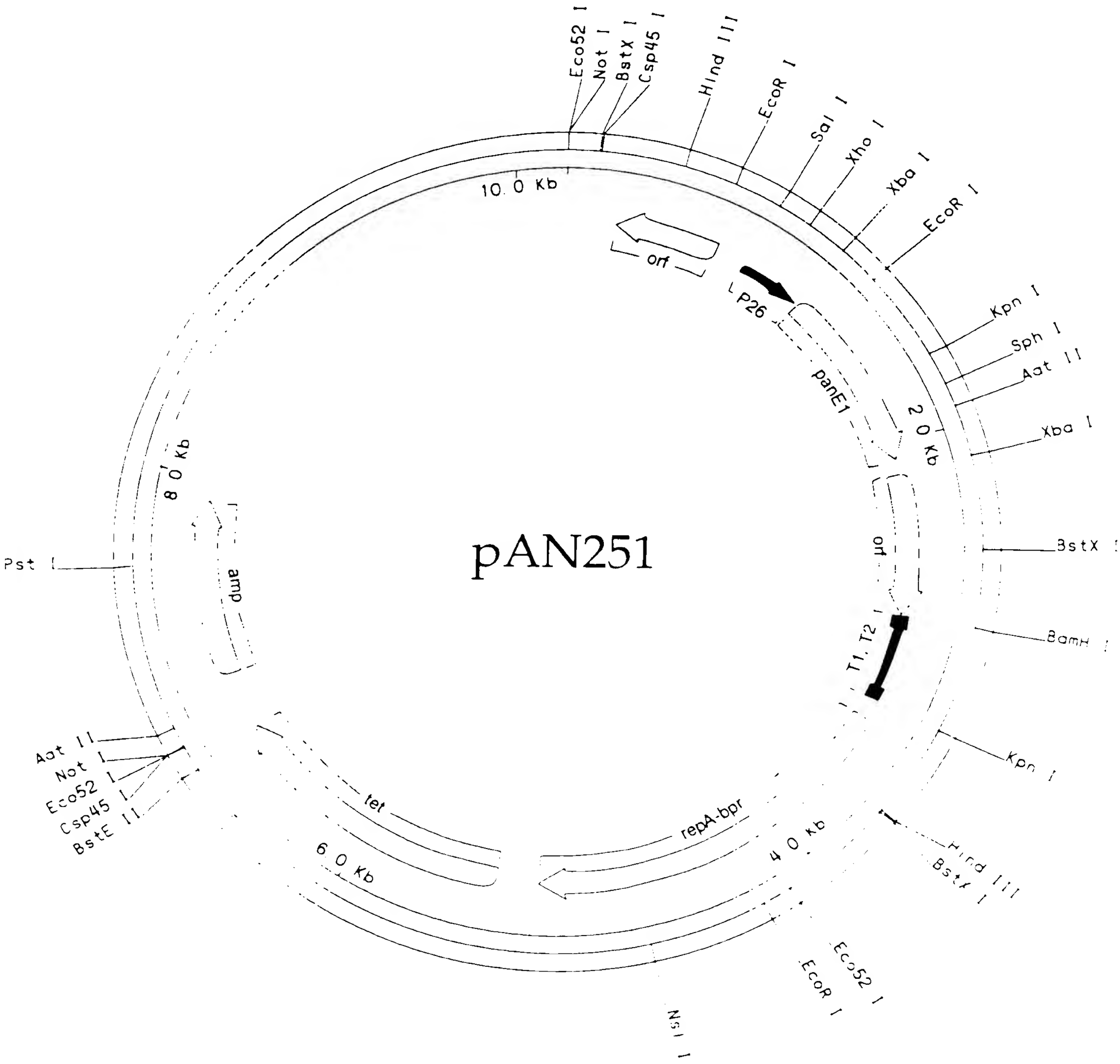


Figure 12. Structure of pAN267, a plasmid designed to stably integrate a P_{26} ilvBNC *cassette at the* amyE *locus.*

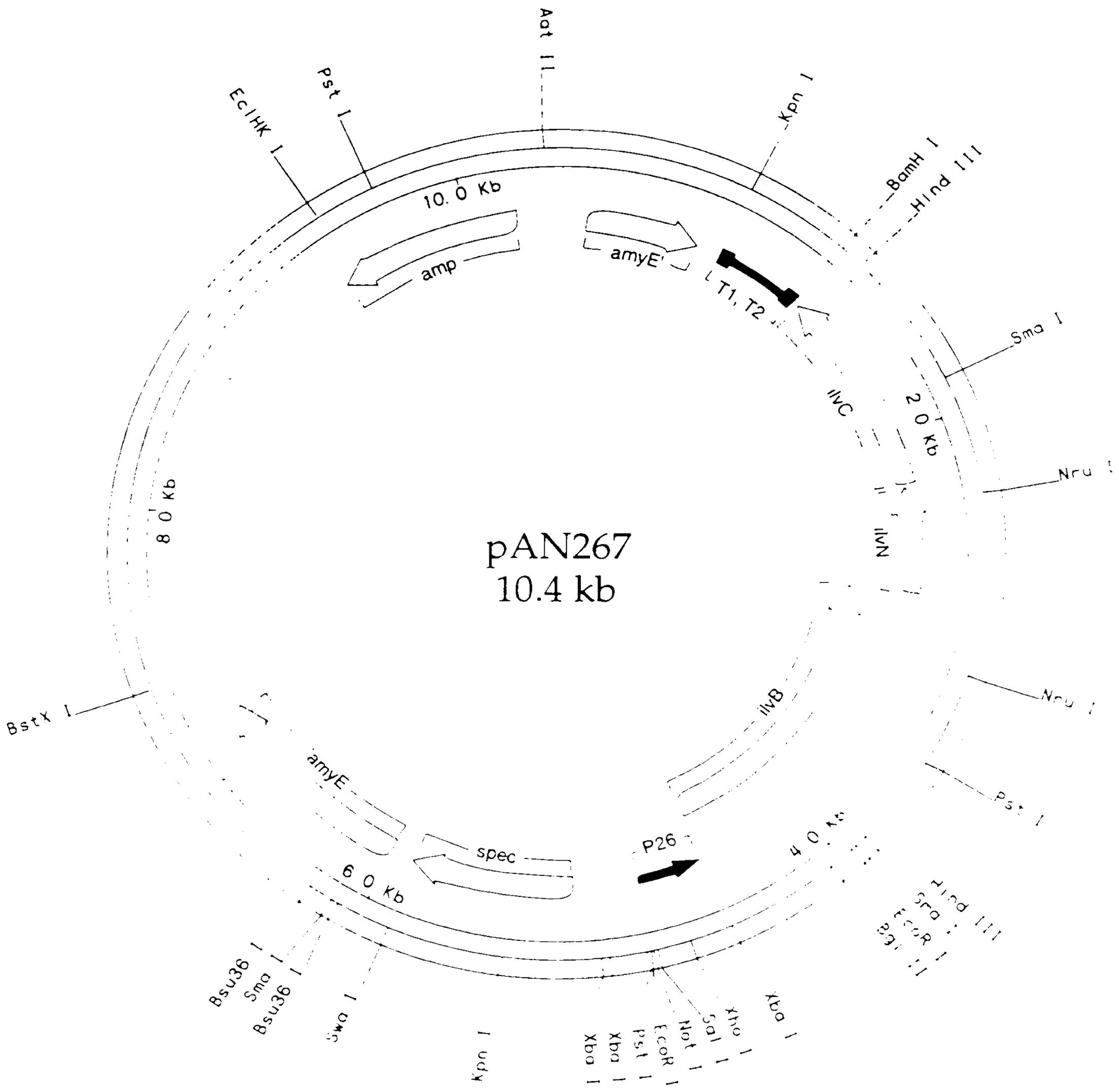


Figure 13 Structure of pAN257, a clone of B. subtilis ilvD *in a low copy vector.*

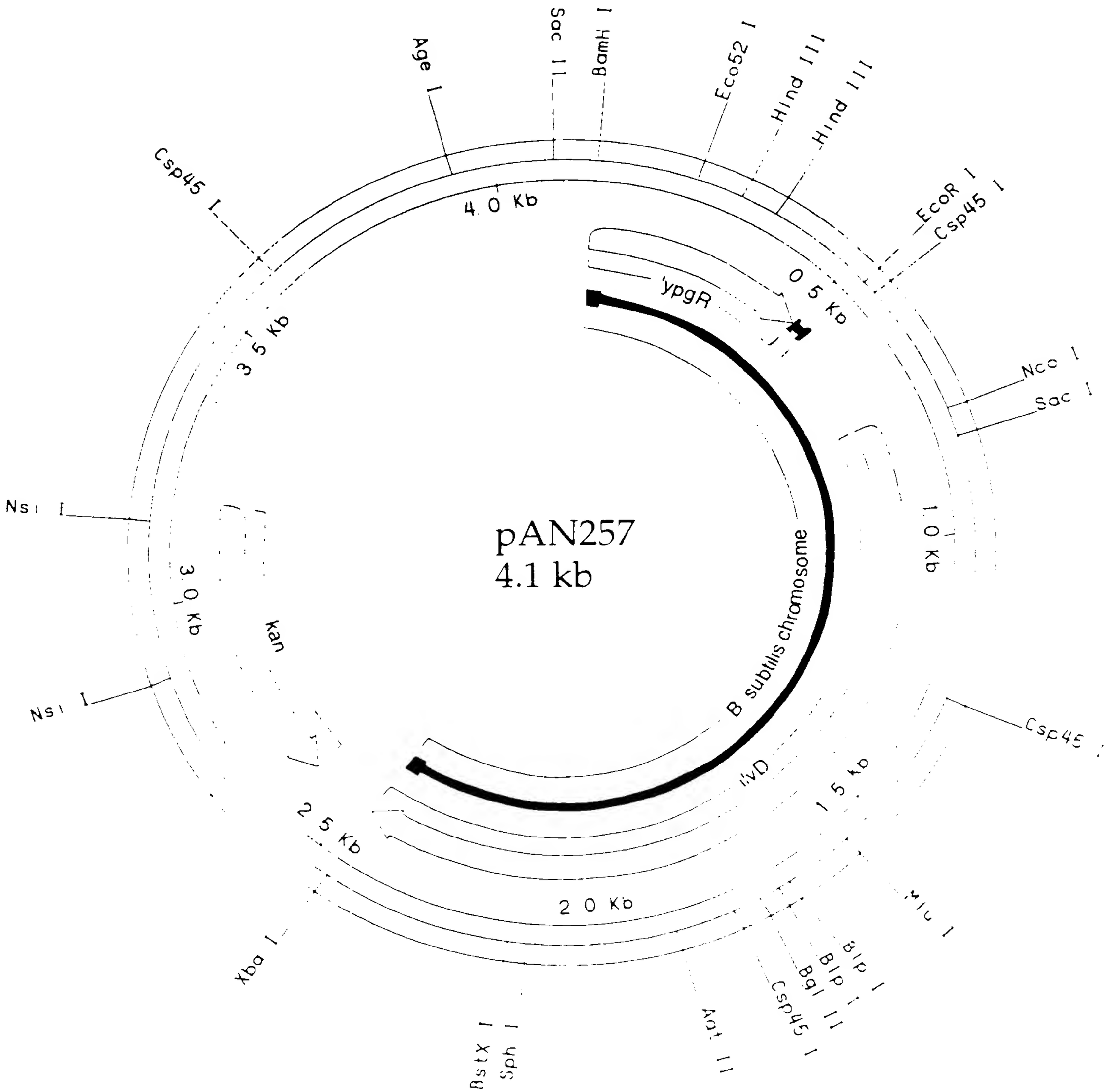


Figure 14 Structure of pAN263, designed to stably integrate a single copy of P_{26} ilvD *at the* ilvD *locus.*

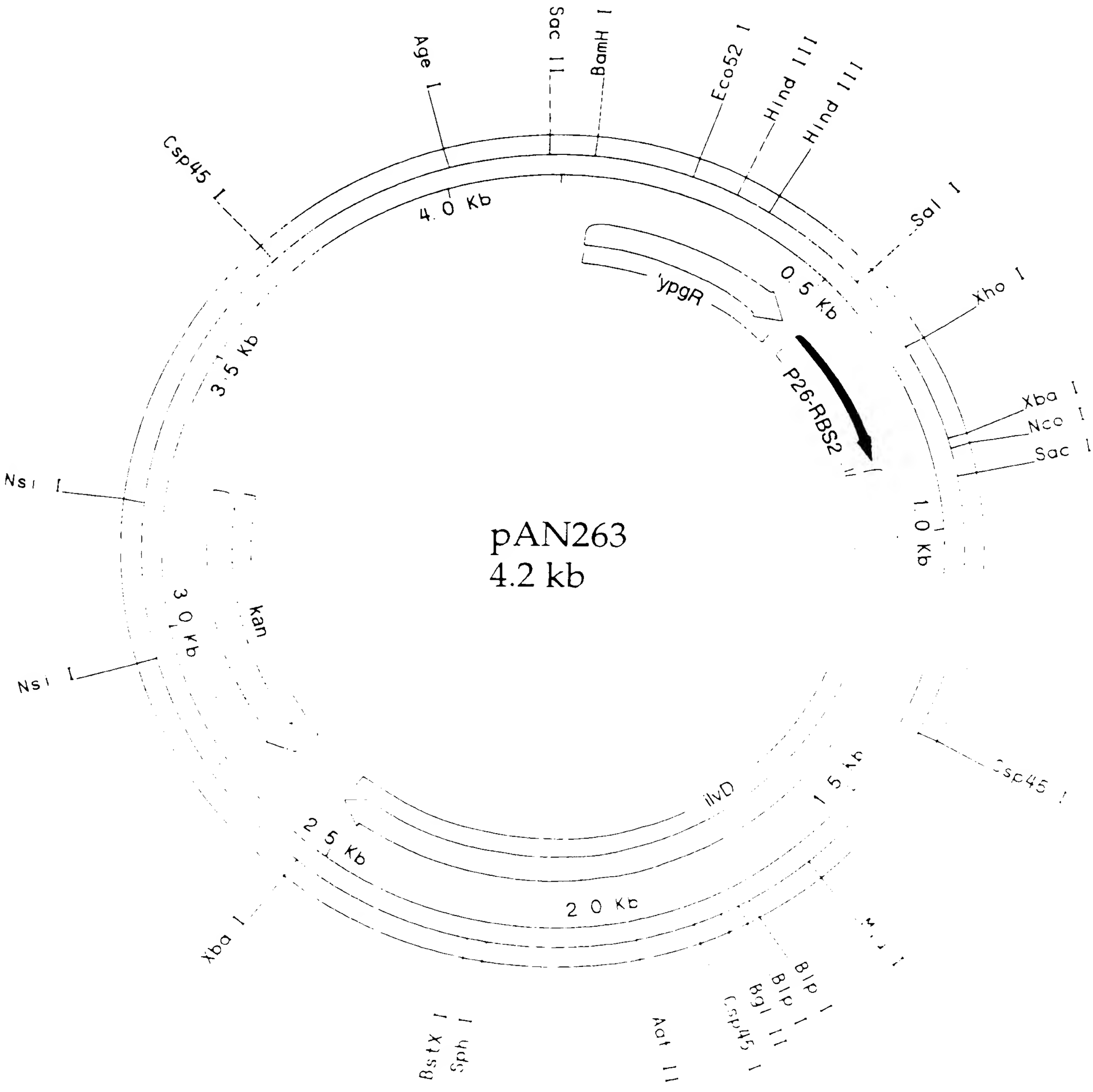


Figure 15 Structure of pAN261, designed to disrupt the B. subtilis ilvD *gene with the* cat *gene.*

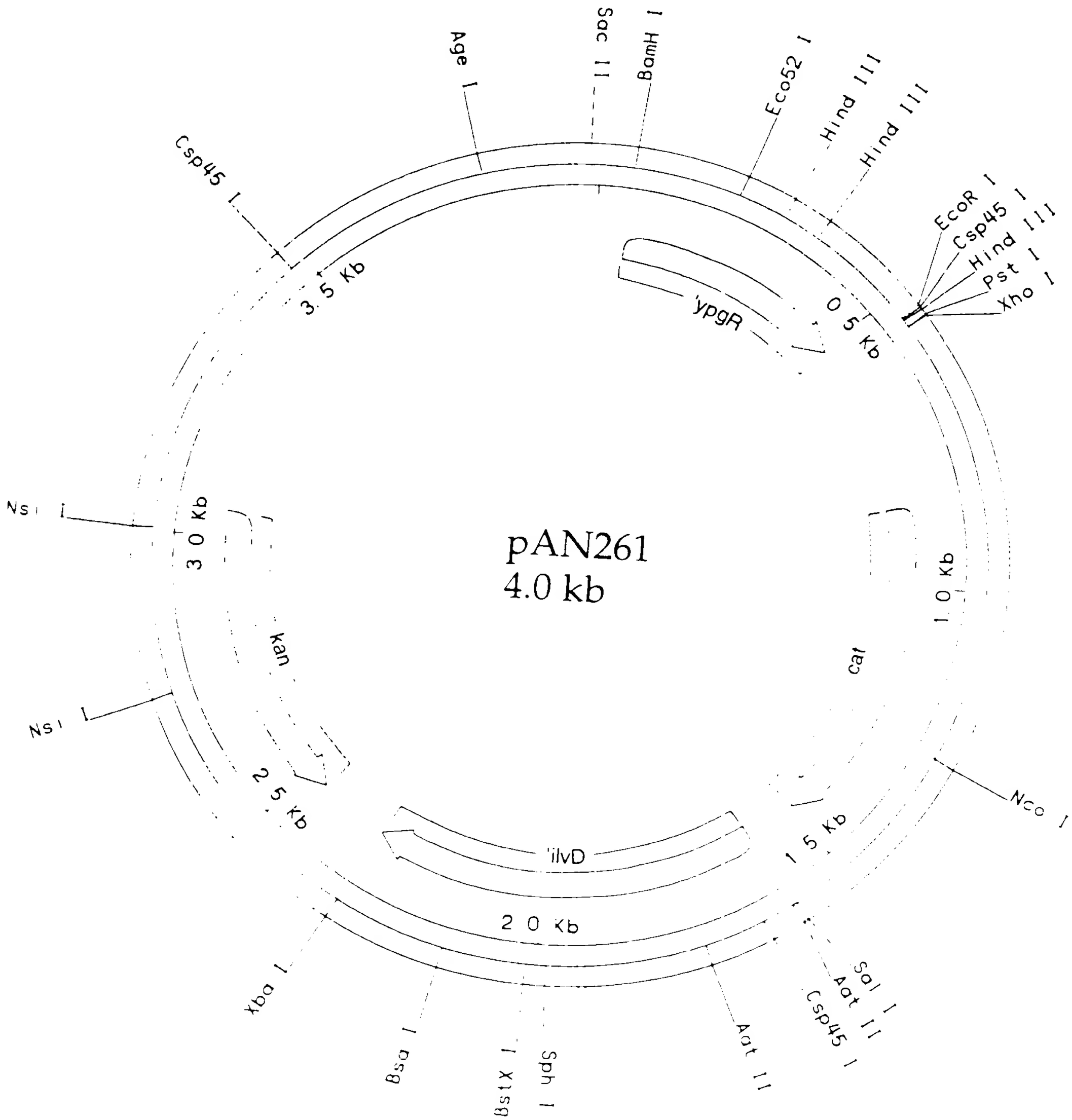


Figure 16

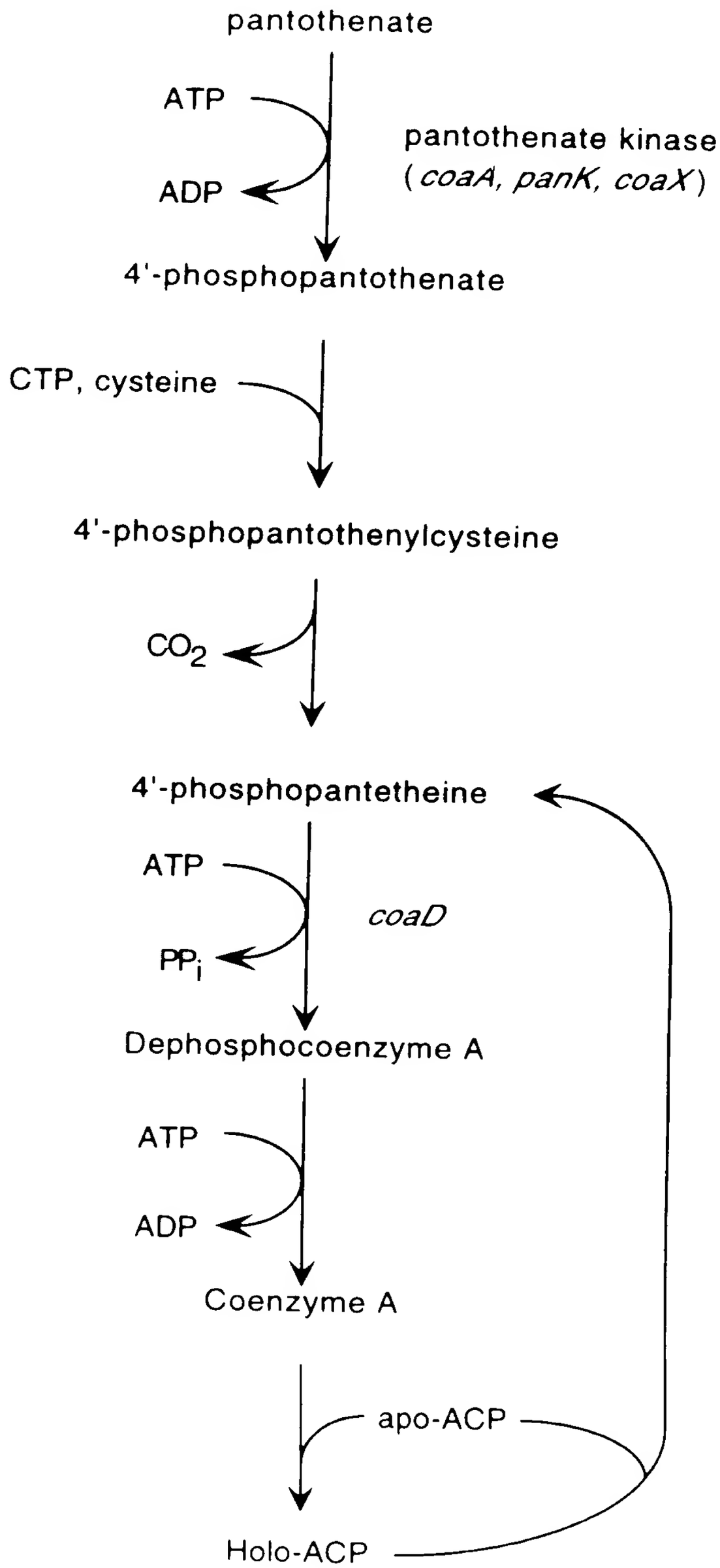

pantothenate
ATP
ADP
pantothenate kinase
(coaA, panK, coaX)
4'-phosphopantothenate
CTP, cysteine
4'-phosphopantothenylcysteine
CO2
4'-phosphopantetheine
ATP
PPi
coaD
Dephosphocoenzyme A
ATP
ADP
Coenzyme A
apo-ACP
Holo-ACP

Figure 17 Structure of pAN296, designed to delete most of the **B. subtilis coaA** *gene and substitute a chloramphenicol resistance gene.*

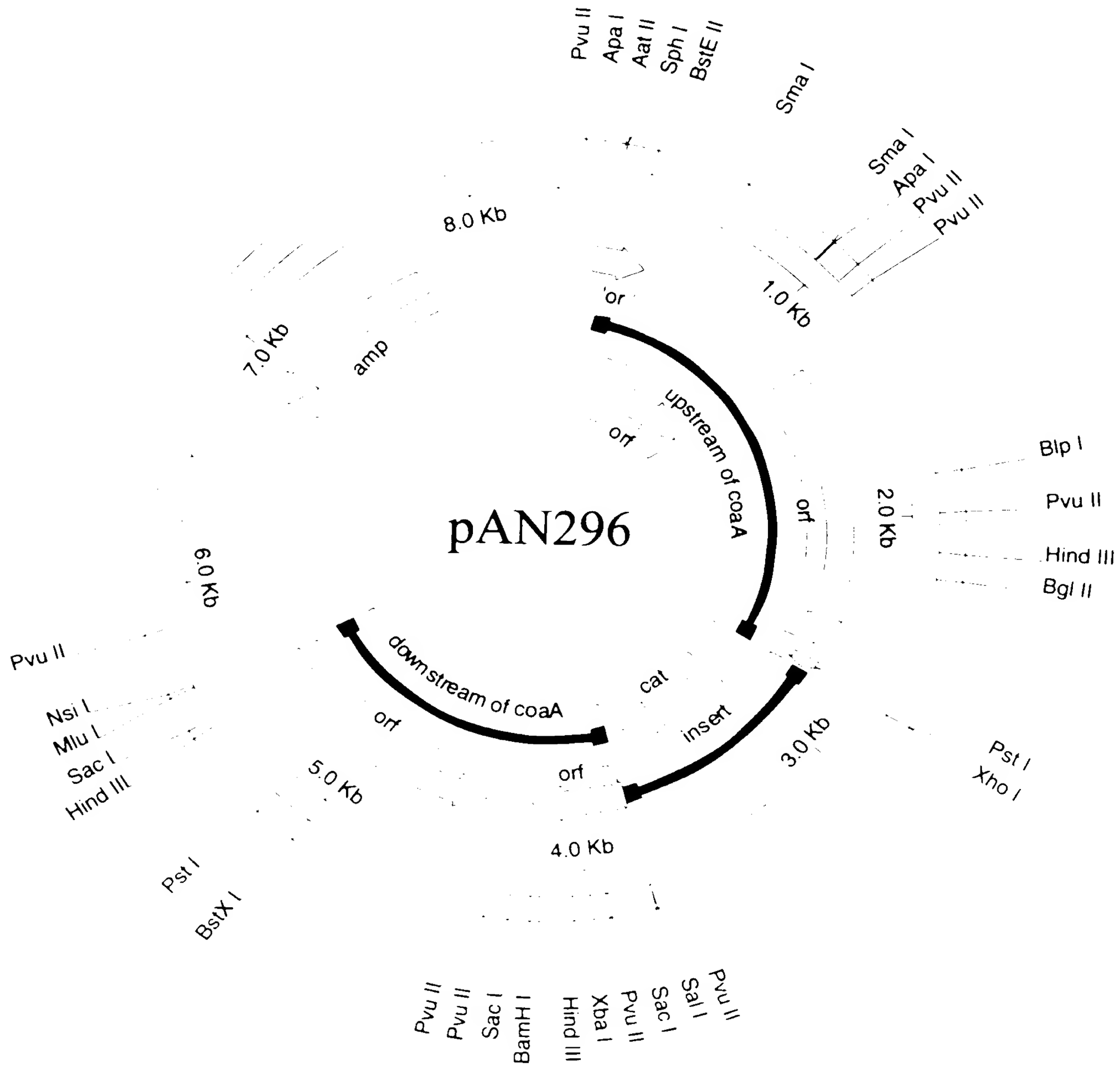


Figure 13 Structure of the B. subtilis *chromosome in the region of the* coaA *gene. The scale is in base pairs and the significant open reading frames are shown by the open arrows.*

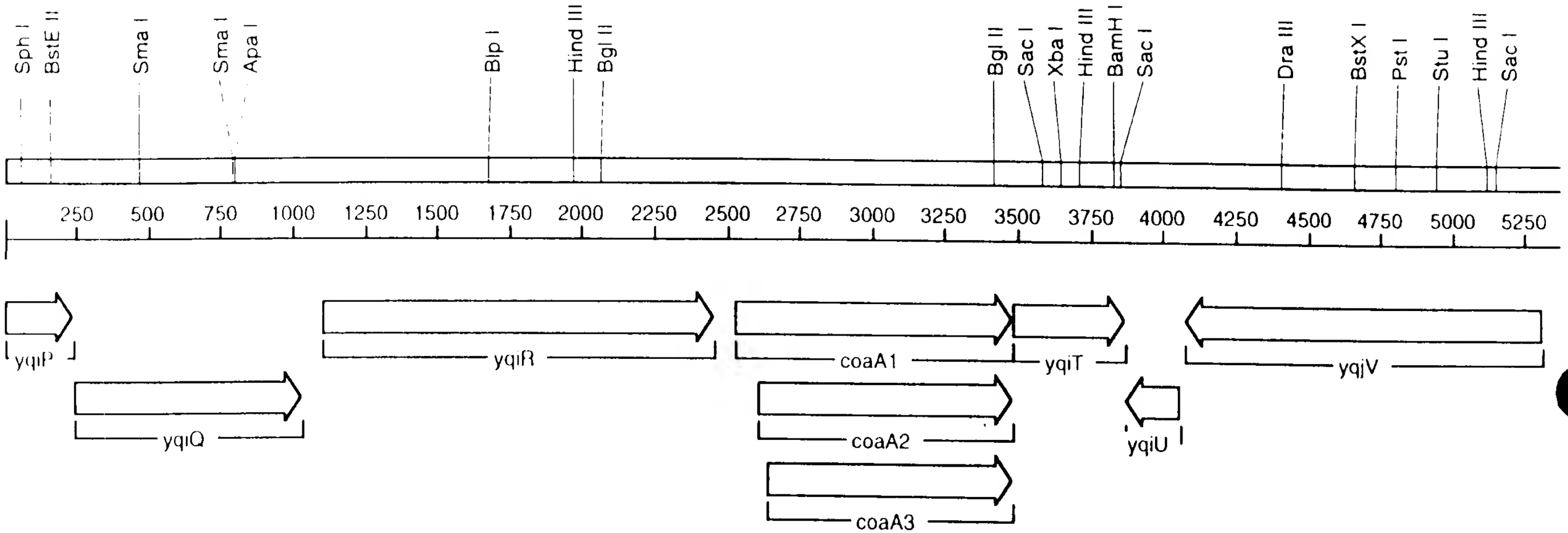


Figure 19 Structure of pAN281, a plasmid for expressing **B. subtilis coaA** *after integration at the* **bpr** *locus. pAN282 and pAN283 have similar structures.*

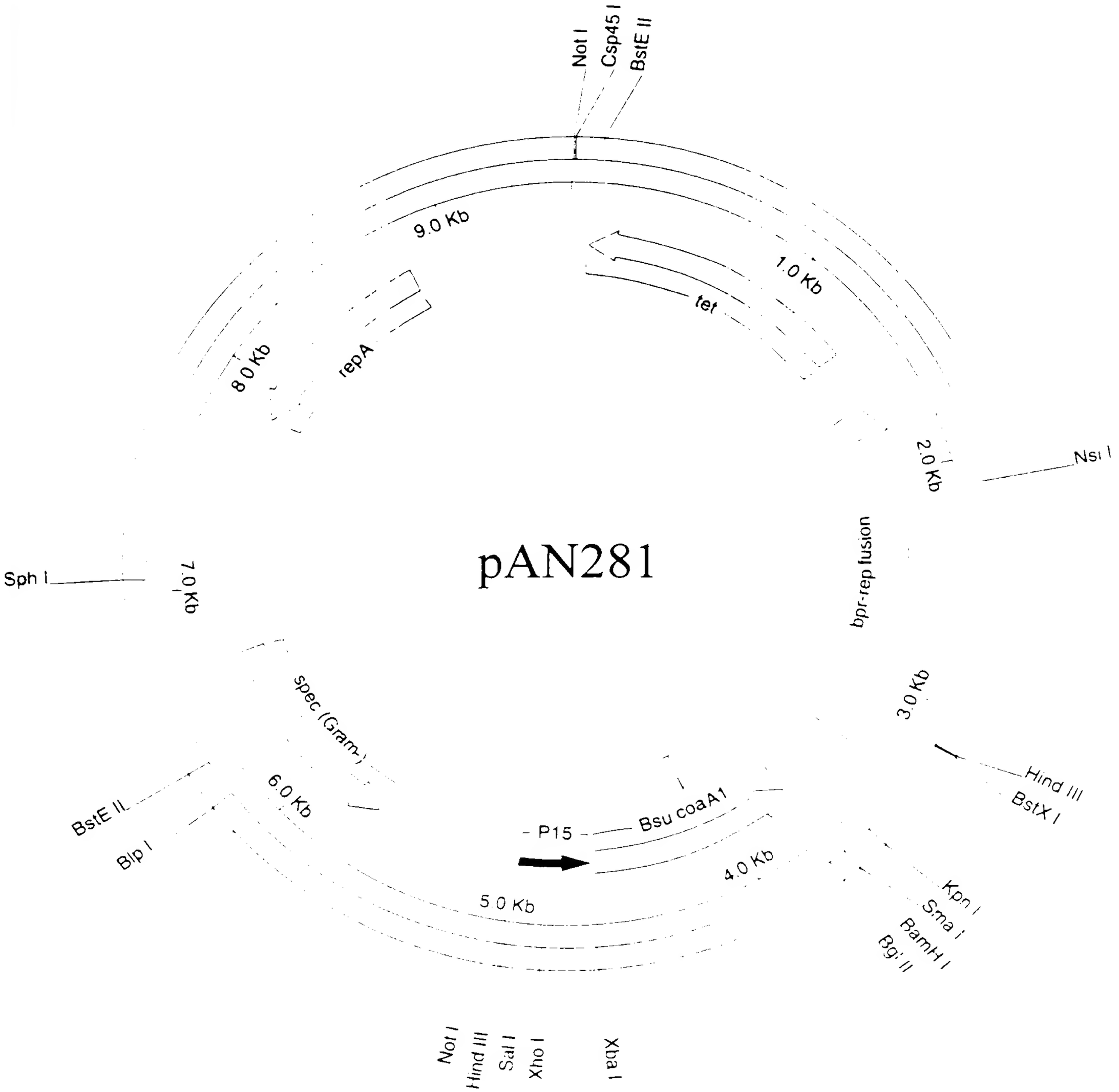


FIG.20A

FIG.20B

Figure 21

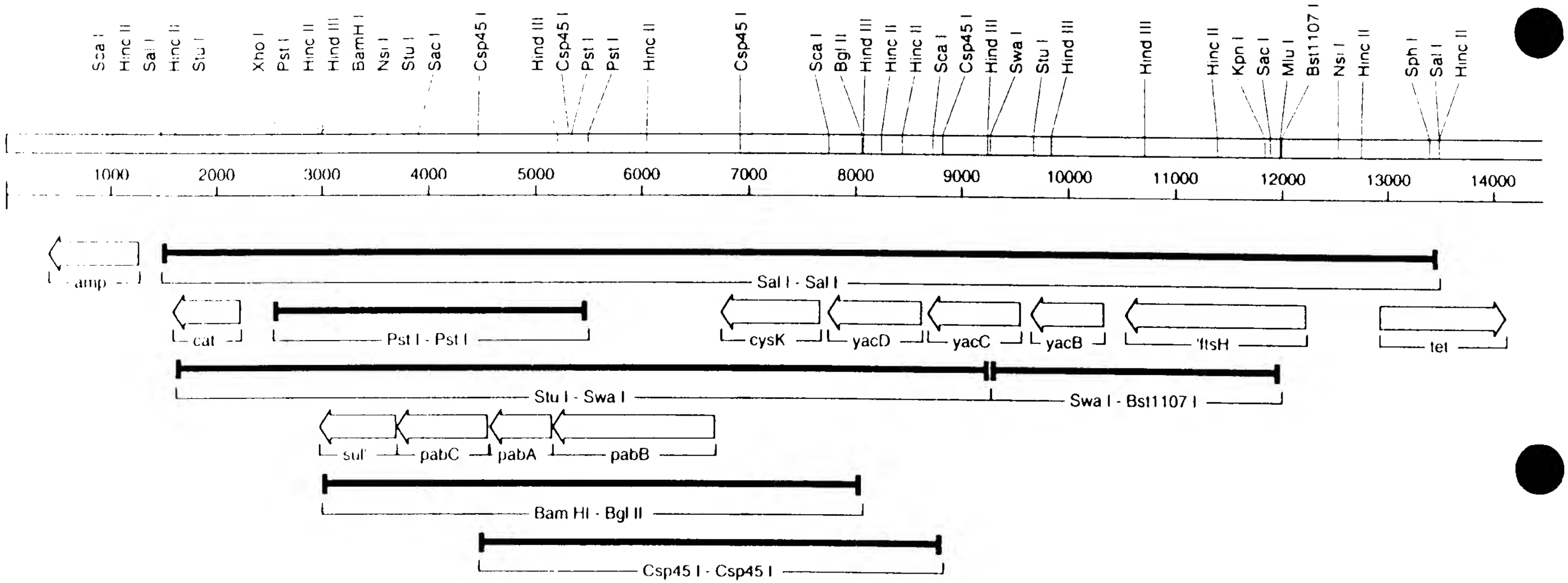

Sca I
Hinc II
Sal I
Hinc II
Stu I
Xho I
Pst I
Hinc II
Hind III
BamH I
Nsi I
Stu I
Sac I
Csp45 I
Hind III
Csp45 I
Pst I
Pst I
Hinc II
Csp45 I
Sca I
Bgl II
Hind III
Hinc II
Hinc II
Sca I
Csp45 I
Hind III
Swa I
Stu I
Hind III
Hind III
Hinc II
Kpn I
Sac I
Mlu I
Bst1107 I
Nsi I
Hinc II
Sph I
Sal I
Hinc II
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
11000
12000
13000
14000
amp
Sal I - Sal I
cat
Pst I - Pst I
cysK
yacD
yacC
yacB
'ftsH
tet
Stu I - Swa I
Swa I - Bst1107 I
sul'
pabC
pabA
pabB
Bam HI - Bgl II
Csp45 I - Csp45 I

Figure 22 Structure of pAN341 and pAN342, two independent PCR- derived clones of **yacB** *(renamed* **coaX***) .*

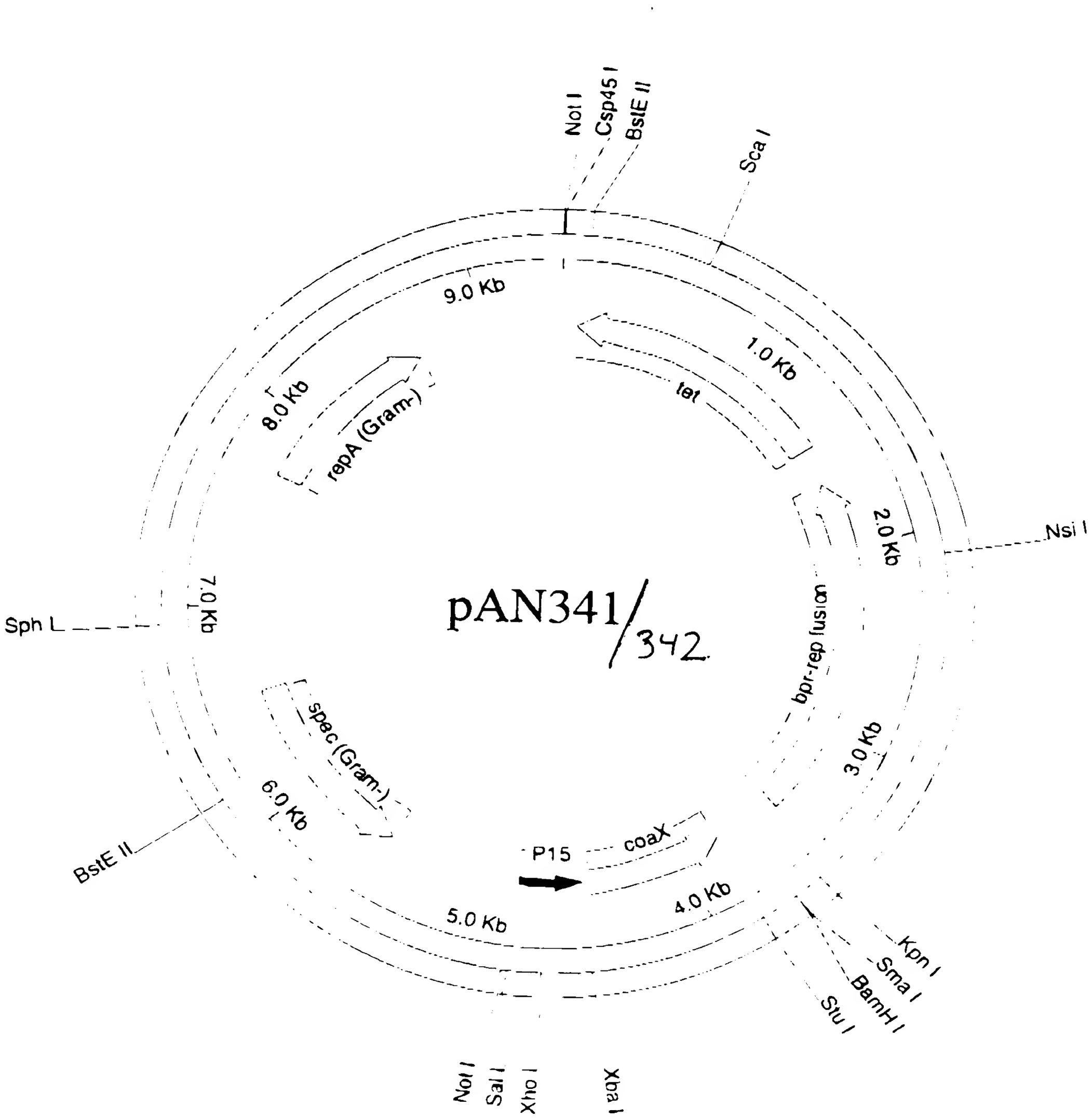


FIG.23A

FIG.23B

FIG.23C

FIG.23D

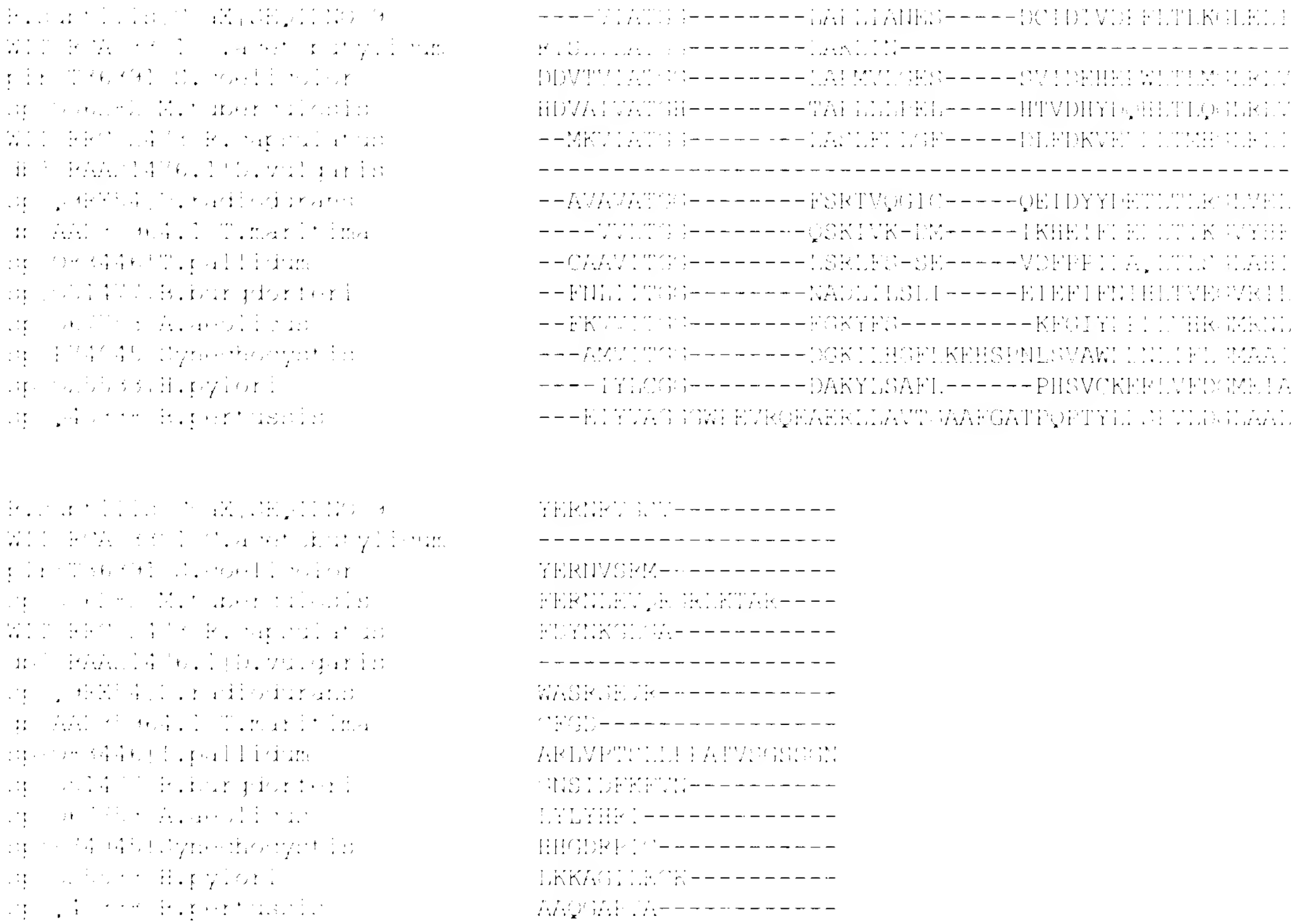


Figure 24 Alignment of a portion of the amino acid sequences of several known or suspected pantothenate kinases. The residues that are mutated in **E. coli coaA15(Ts)** *and* **B. subtilis coaA** *from plasmid pAN282A are indicated below and above the alignment, respectively. The coordinate given in the left margin for the* **B. subtilis** *protein refers to the* **coaA1** *open reading frame.*

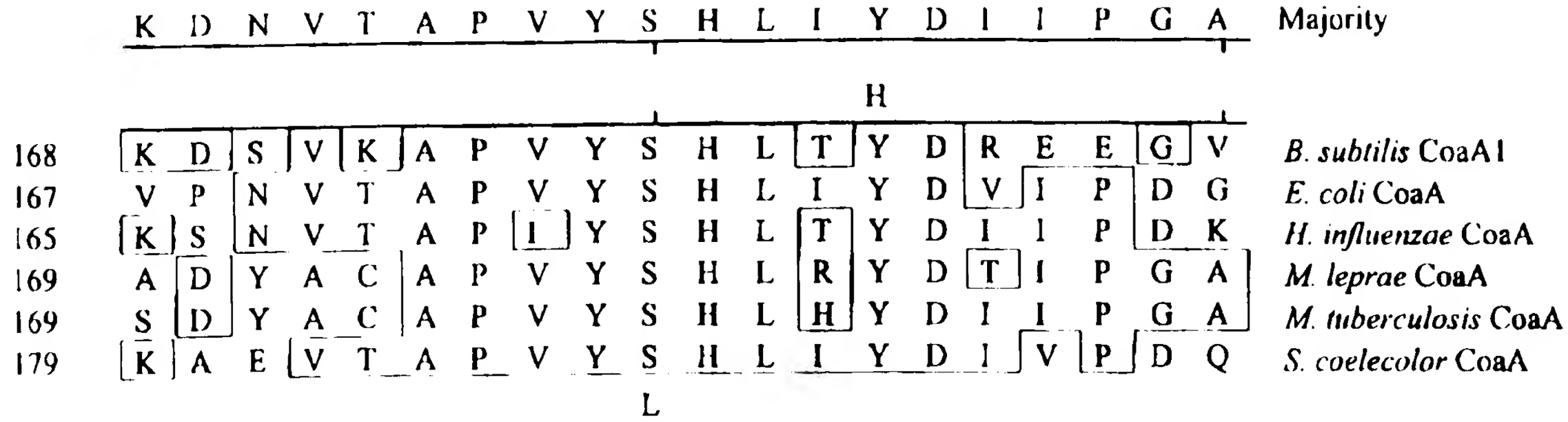


Figure 25 Structure of pAN294, a plasmid for integrating mutagenized **B. subtilis coaA** *at its native locus.*

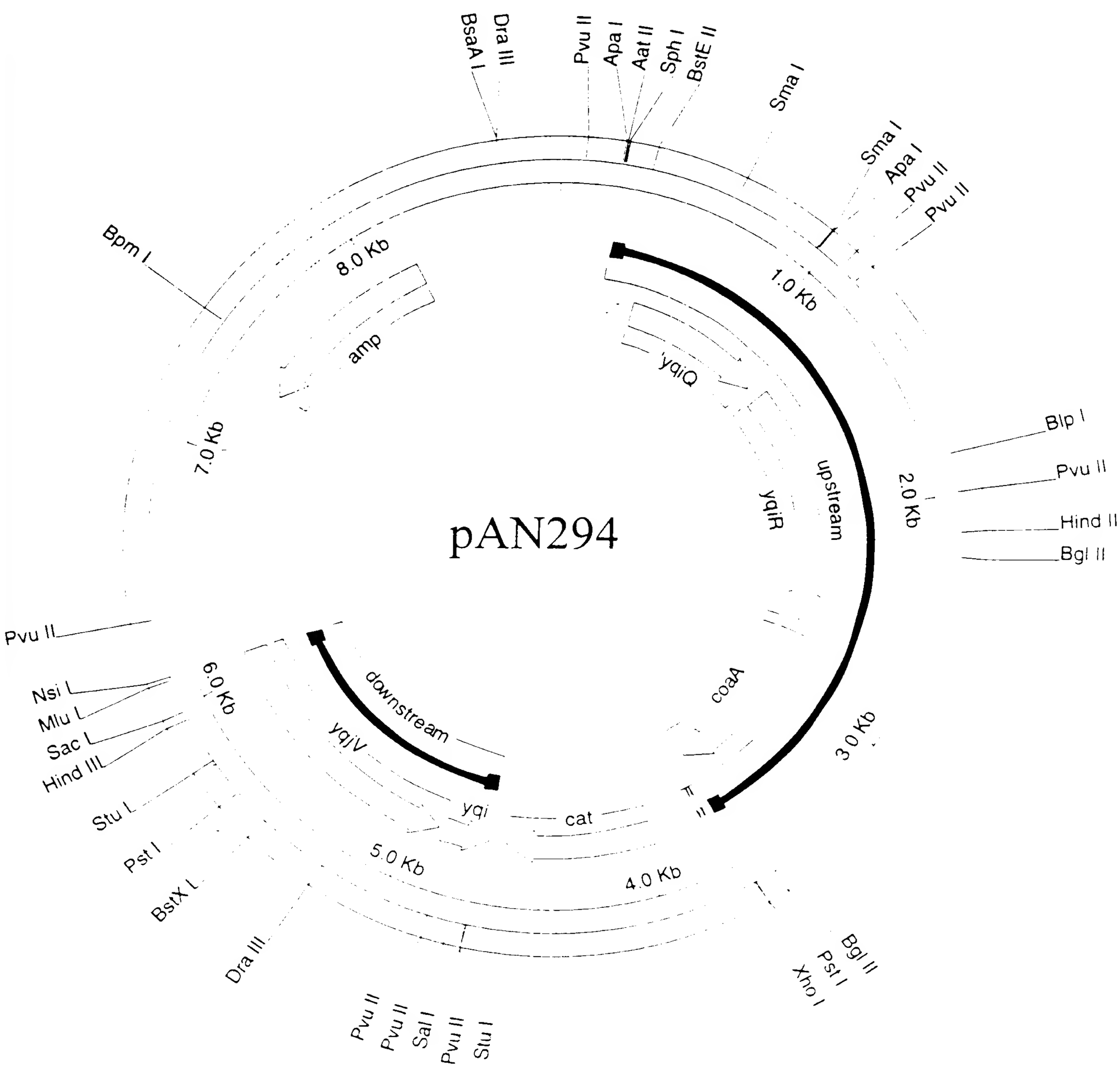


Figure 26 Structure of pAN336, a plasmid designed to delete **B. subtilis coaX** *from the chromosome and replace it with a kanamycin resistance gene.*